March 30, 1971

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Dear Mike,

I had wanted to write to you before but England's postal strike (which we kept hearing was about to end but never did) really fouled things up.

First off let me say that I hope things are going well at ICRF. I saw Howard Hiatt about ten days ago and he told me he'll be visiting you in London shortly, so you'll get our regards from him. George Stark was back for a few days' visit a month ago and he filled us in on some of the things going on at ICRF or at least what's going on in his project with Lionel. I've not heard too much from Bill Folk directly but George says he's doing pretty well.

We've been trying to go full blast on the construction of SV40 dI:dC hybrid molecules and I'm reasonably sure now we can do it. Hopefully, we'll have the first ones in a few months time if not sooner. As things have gone on, many new possibilities for their use have become apparent and these have gotten us even more excited. Letters are such a bad way to tell you about them, that I look forward to seeing you soon to tell you about the new developments. Which brings me to one of the reasons for this letter: I heard you will be coming to the States shortly; is that correct? If so, can we hope to get you to visit Stanford? Please let me know when and if you will be on the west coast. And, if Veronica comes with you, we'd love to have you both spend a bit of time in the San Francisco Bay area. Certainly a bit more than you did last time. San Francisco is not London, but it has its interesting features which we'd like to show you. I've also seen your name on the program of the Cancer Gordon Conference in late August-September. Howard asked Millie and me to come East to spend some time with them after the meeting and perhaps see a bit of Martha's Vineyard. If you're there, we could have a reunion.

I assume George told you about where we are on our joint project now. Enclosed is a curve of our annealing kinetics done using our technique of following the rate of annealing of sheared, denatured PY DNA (single-strand lengths of about 400-500 bases).

Instead of using the laborious hydroxyl apatit column method for measuring the proportions of single- and double-stranded DNA at each time point (which has the uncertainty of not knowing how a partially annealed molecule behaves) we've worked out the conditions for using any one of three single-strand specific DNases to degrade all unpaired bases. Starting with ^{32}P sonicated, denatured PY DNA (of the order of 10^{-3} to 10^{-5} A_{260}) in the presence or absence of a large excess (2000-fold of salmon sperm DNA) >95% of the ^{32}P DNA is converted at zero time to acid-soluble material in 45-60' with any of the enzymes we use. As annealing proceeds, the ^{32}P DNA becomes resistant to degradation by the nuclease and when annealing is complete, 80-90% (should be 100%) of the ^{32}P is resistant to degradation. As you would expect the rate of annealing is proportional to the amount of ^{32}P-DNA we start with and the ionic strength. With a given amount of ^{32}P-DNA the Cot value at a particular ionic strength and temperature is characteristic. If unlabeled PY-DNA sheared to the same extent is added, the annealing time is shortened (the Cot value decreases) and the change in the Cot of value is a measure of the amount of unlabeled PY DNA added. Shortly, we shall begin to do experiments with the sheared-denatured DNA from the stable and abortively transformed cells we isolated last summer to see if we can detect viral DNA sequences in the cell DNA.

There is only one fly in the ointment. To make the assay sensitive and to use as little of the cellular DNA as possible, we want to put in as hot and as little of the ^{32}P-DNA as we can. We've solved the problem of making very hot ^{32}P-DNA (hopefully 5×10^6 cpm/μg or higher) but that's only part of it. It's true that the hotter the DNA the less we need to use and the greater the sensitivity. But the less we use the slower the annealing rate so that when one gets to 10^{-5} A_{260}/ml the Cot value (or better the annealing time) is very low even at the highest ionic strength we can use. So we have to compromise. The higher the amount of ^{32}P-DNA we use, the more cell DNA we need to find traces of viral DNA. The lower the amount of ^{32}P-DNA, the more sensitive the measurement but the longer the annealing times. Hopefully, we'll have that compromise worked out soon. But the method looks quite good for measuring viral DNA replication or for tracing the movement or presence of viral DNA without having to label it.

I'm very busy now organizing and giving a course on Tumor Viruses as Probes for Animal Cell Regulatory Mechanisms. It means two, two-hour sessions per week for 10 weeks. If I survive, it will have been a very good experience for me. But with all the other administrative load and trying to keep up with all that's going on in the lab, its a trying period. I look forward to the summer for peace and quiet and a chance to work in the lab.

There's a chance I might be through London in July if I go to Erice, Sicily for that school I agreed to go to a year ago. That's not yet certain, but if I do, and you are there, I'd certainly like to stop by.

With best regards to all,

Sincerely,

PB/i